The clinical significance of allelic alteration of TSG loci in cervical intraepithelial neoplasia

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The involvement of the HPV in the development of cervical cancer has been firmly established. Specific human papillomavirus (HPV) types appear to be necessary etiological factors for most cervical cancers. High-risk HPVs such as HPV16 and HPV18 are causative agents for high-grade intraepithelial neoplasia (CIN3) and cervical cancer. One mechanism by which the virus contributes to disease progression is by causing genetic instability of the host genome [Hashida et al 1992; White et al 1994]. This is explained in part by the interaction of the viral oncoproteins E6 and E7 with key cell regulatory proteins such as p53 and pRb thereby deregulating the cell cycle, cell differentiation, DNA repair, and apoptosis [Scheffner et al 1990; Mietz et al 1992; Dyson et al 1989; Sherman et al 1996; Phelps et al 1992]. Because HPV infection does not always lead to cervical cancer, other genetic alterations must also play a role in tumor development. Moreover, epidemiological data and experimental studies demonstrate clearly that infection *per se* does not suffice to induce malignancy. Additional genetic alterations seem to be required for their development and progression.

Cervical carcinomas develop as a result of multiple genetic alterations, and specific alterations lead to specific clinical behavior. However, the effect of such alterations on the occurrence and progression of preinvasive cervical cancer remains unknown. A loss of heterozygosity (LOH), which points to a role for tumor suppressor genes (TSGs), oncogene amplification, and point mutations, are all thought to be involved, but there is as yet no complete picture of the relative roles for each of these genetic changes in patients with cervical carcinomas. To play a role in tumorigenesis, both copies of a TSG must be inactivated. The loss of one allele in a chromosome region may point to the presence of a TSG in that region. Several studies have shown that LOH at specific chromosomal sites is frequently associated with the recurrence of various cancers, *e.g.*, 13q14.3 in oral carcinoma [Ogawara et al 1998], 10q in human lung cancer [Petersen et al 1998], and 11p15 in breast cancer [Karnik et al 1998]. Although cytogenetic studies of cervical cancer are relatively few, they have revealed frequent, nonrandom chromosomal changes [Atkin et al 1990]. Studies of LOH in patients with cervical carcinoma have also reported a high frequency of allelic deletions affecting 3p [Jones et al 1992; Kohno et al 1993], 5p [Mitra et al 1995], 17p [Jones et al 1994; Mitra et al 1994; Mullokandov et al 1996; Harima et al 1999], and 18q [Kersemaekers et al 1998b]. A LOH on chromosome 6p has also been reported in patients with cervical carcinoma Mitra et al 1994; Mullokandov et al 1996; Rader et al 1996; Kersemaeker et al 1998a]. However, the importance of LOH on chromosome 6p in the recurrence of cervical cancer after radiotherapy remains unknown.

It has been shown previously that a significant number of invasive cervical cancers have nonrandom chromosomal losses in 3p, 6p, 10p, 11q, 2q, 6q, and 19q, thereby suggesting that genes involved in the suppression of tumor development or progression are located in these regions (Rader et al 1996). Among these genetic alterations, chromosome arm 6p is one of those most frequently involved in a loss of heterozygosity in patients with cervical carcinoma [Chatterjee et al 2001]. Cervical intraepithelial neoplasia III is considered the precursor lesion for invasive carcinoma of squamous type. In CIN III, the most frequent allelic lass was found in 3p and 6p. In addition, by using several derivatives of chromosome 10 for further fusion experiments, the chromosomal region responsible for senescence could be assigned to 10p14–p15. The potential significance of loss of gene function in this region is underlined by the high frequency (38.7%) of loss of heterozygosity in cervical cancers including early stage tumors [Poignee et al 2001].

On the other hand, the reason why not all human papillomavirus (HPV)-positive high-grade lesions of the cervix progress to cancer is not understood. Storey and colleagues [Storey 1998] showed that polymorphisms in codon 72 of p53 could determine the efficiency of HPV 16 or HPV 18 E6 in degrading p53 in vitro. These data were further supported by testing cervical cancer biopsy specimens from UK women, which showed a seven-fold enrichment of the arginine allele over the proline allele. Several groups have failed to confirm this result [Rosenthal 1998, Lanham 1998, Hayes 1998, Josefsson 1998]. In the latter reports no association of the p53 codon 72 arginine with cervical cancer was found. Moreover,the proportion with codon 72 arginine in the healthy controls was considerably higher in these reports than in the study of Storey and colleagues [Storey 1998]. However, in a recent article,Zahbe et al. [zahbe 1999] found that the p53 arginine polymorphism represents a potential risk for cervical cancer development, consistent with the concept proposed by Storey and colleagues. In other word, discrepancy between these studies still exists and the reason needs to be clarified.

The term atypical squamous cell of undetermined significance (ASCUS) describes a minor degree of nuclear pleomorphism limited to the basal layers of cervical epithelium in the absence of severe inflammation with associated normal mitoses, koilocytosis, or koilocytosis associated features. Cervical intraepithelial neoplasm grade 1 means a dysplasia lesion involves the several layers of cell at lower third squamous epithelium. From a corroborative study-- Laboratories enrolled in the College of American Pathologists Interlaboratory Comparison Program in Cervicovaginal Cytology,, it was found that median reporting rates for epithelial abnormalities were as follows: ASCUS, 4.5%; low-grade squamous intraepithelial lesion (low-grade SIL), 1.6% [Davey et al 2000].

The prognosis in cervical epithelial changes of uncertain significance was found to be similar to that of CIN1 [Heatley 2001]. Therefore, Women with ASCUS or CIN 1 who are followed up regularly are at low risk for development of invasive cancer [Rabb et al 1999; Melnikow et al 1998]. Assessment of cytologic follow-up for patients with atypical squamous cells of undetermined significance or low grade squamous intraepithelial lesions may be regarded as the standard recommended management [Alanen et al 1998]. Women with ASCUS or CIN 1 who are followed up regularly are at low risk for development of invasive cancer [Rabb et al 1999; Melnikow et al 1998].

Recently, it was found that 17-18% of ASCUS was stable or progressed. [Giudice et al 2000]. However, which factors that can predispose the lesion to progress are still unknown. Most recent international or domestic studies did not focus on this issue. In a recent report that studied 52 eligible patients having conizations or hysterectomies as their histological outcomes and were tested for loss of heterozygosity, it was found that use of loss of heterozygosity in at least one locus was useful for predicting presence of high-grade cervical neoplastic lesion in the conized specimen [Chang et al 2001]. In fact, predictors of persistent and regressed disease for ASCUS or CIN1 were not identified [Duggan et al 1998].

In our department, we do not do any surgical operations (such as conization) to the cases with ASCUS or CIN. It seems that our management is the same as that recommended recently worldwide [Alanen et al 1998]. Assessment of cytologic follow-up for patients with atypical squamous cells of undetermined significance or low grade squamous intraepithelial lesions may be regarded as the standard recommended management [Alanen et al 1998].

Because of that

1). women with ASCUS or CIN 1 who are followed up regularly are at low risk for development of invasive cancer [Rabb et al 1999; Melnikow et al 1998], and

2). prognosis in cervical epithelial changes of uncertain significance was found to be similar to that of CIN1 [Heatley 2001], and

3). assessment of cytologic follow-up for patients with atypical squamous cells of undetermined significance or low grade squamous intraepithelial lesions may be regarded as the standard recommended management [Alanen et al 1998], and early colposcopy is suggested to be the clinical policy to exlude high-grade lesions [Melnikow et al 1998], and

4). predictors of persistent and regressed disease for ASCUS or CIN1 were yet not identified [Duggan et al 1998], even after reviewing the domestical or international literatures up to the present time, we dare to decide to conduct this study.

In this prospective study, we intend to identify the methods which may be helpful to determine or predict the progress or regress of these early cervical lesions. The cases with ASCUS or CIN1 on Pap smear were followed up without doing conization or hysterectomy and will do a long term follow up. This study experients will include HPV status, and p53 polymorphisms, as well as the genetic alterations which will involve the loci that most frequently found to occur.

CIN introduction

Carcinoma of the uterine cervix is the second most common malignancy of women worldwide in both incidence and mortality[Pontén et al 1995; NIH]. Papanicolaou (Pap) smear screening is the most effective tool currently available for early detection, leading to a greater than 70% reduction in cervical cancer mortality since the test was introduced 50 years ago. However, the Pap smear is not a perfect test; it has a high false-negative rate (variously estimated at 2% to 40%), due to a combination of sampling error, processing artifacts and the nature of subjective interpretation[NIH, Larsen 1994]

HPV

The involvement of the HPV in the development of cervical cancer has been firmly established. Because HPV infection does not always lead to cervical cancer, other genetic alterations must also play a role in tumor development. Specific human papillomavirus (HPV) types appear to be necessary etiological factors for most cervical cancers. Nevertheless, additional genetic alterations seem to be required for their development and progression.

HPV and CIN

Certain human papillomavirus genotypes are etiological agents in the development of cervical carcinoma [Our Hansen 1991] HPV16 is the most frequently detected genotype in invasive cervical carcinoma as well as in cervical intraepithelial neoplasia (CIN) I-III. HPV 18 is also reported to be related with these lesions. Persistent HPV infection is a risk factor for the progression of a preinvasive lesion to invasive cancer. However, most high-risk HPV infections do not progress to cancer. Epidemiological studies have identified additional risk factors that may contribute to the development of cervical carcinoma. These include age at infection, smoking, hormonal factors, genetic predisposition, and immune response. [Schiffman 1993]

Thus,the tumour biology of cervical intraepithelial neoplasia and cervical cancer is unusual. A large variety of individually distinct forms crudely divided into slight, moderate, severe dysplasia

and carcinoma in situ exist. Virtually all contain genital human papillomavirus either as infectious virions or as episomal or integrated DNA. A proportion of infected women develop condyloma, precancer and subsequently, in a minority, invasive cancer. Risk of precancer is statistically related to infection with genital HPV, but differences in risk between populations with high and low prevalence of HPV are larger than expected from a direct correlation. Findings fit with HPV as a major risk factor, but other factors must also be operative.

High risk HPV, typically 16 or 18, is preferentially associated with high grade dysplasia and in situ cancer either because it increases risk of clonal progression to these forms or induces them de novo. Severe dysplasia, in situ and invasive cancer always present as monoclonal lesions. Spontaneous mutation rate and physicochemical carcinogens seem insufficient for the creation of a malignant phenotype in cells of the transformation zone. Currently HPV is the only strong candidate for such a feat. Any or all of the following mechanisms may play a role: overexpression of viral E6 and E7 genes, often triggered by disruption of control elements upon integration of viral DNA into the cellular genome, activity of specific (E6) configurations in certain HPV variants, inactivation of TP53 with decreased capacity for DNA repair and enhanced likelihood of accumulation of "transforming" mutations and viral integration at sites controlling function of cellular oncogenes and/or suppressor genes. Low risk types are almost always associated with squamous differentiation, HPV 16 usually also with squamous differentiation and HPV 18 with adenosquamous or adenomatous differentiation.

Definition of ASCUS and CIN1

The term atypical squamous cell of undetermined significance (ASCUS) describes a minor degree of nuclear pleomorphism limited to the basal layers of cervical epithelium in the absence of severe inflammation with associated normal mitoses, koilocytosis, or koilocytosis associated features. Cervical intraepithelial neoplase grade 1 (CIN1) means a dysplasia lesion involves the several layers of cell at lower third squamous epithelium. The new Bethesda System terminology has opened a series of problems about the ASCUS and Low-Grade Squamous Intraepithelial Lesion categories, particularly on their treatment and follow-up.

Interlab difference of ASCUS

However, there are interlaboratory or interobservor difference in the rate of ASCUS. In a recent study, a cytologic diagnosis of ASCUS were reviewed independently by 5 experienced pathologists [Grenko et al 2000]. Agreement was better performed for high-grade squamous intraepithelial lesions (HSIL) and low-grade squamous intraepithelial lesions (LSIL) compared to those for ASCUS. Intraobserver reproducibility in the interpretation performed for ASCUS ranged from poor to excellent. They conclude that variability in the interpretation of biopsy specimens plays an important role in the differences in rates of dysplasia reported for the follow-up of ASCUS. [Grenko et al 2000]

Incidence of ASCUS

In a corroborative study-- Laboratories enrolled in the College of American Pathologists Interlaboratory Comparison Program in Cervicovaginal Cytology, 768 laboratories returned the 1997 questionnaire focusing on atypical squamous cells of undetermined significance (ASCUS) and glandular cells of undetermined significance (AGUS).The study found median reporting rates for epithelial abnormalities were as follows: ASCUS, 4.5%; AGUS, 0.3%; low-grade squamous intraepithelial lesion, 1.6% [Davey et al 2000].

ASCUS and CIN1-- the same treatment

The optimal management of low grade Papanicolaou (Pap) smear abnormalities remains controversial.

In a recent study, it was found that the prognosis in cervical epithelial changes of uncertain significance is similar to that of cervical intraepithelial neoplasia grade 1 (CIN1) [Heatley 2001]. In his study, the slides from 128 women with low grade cervical abnormalities, accessioned consecutively, were reviewed. In 43 women the initial diagnosis of ASCUS was confirmed and in 30 women the initial diagnosis of cervical intraepithelial neoplasia grade 1 was confirmed. Comparison of follow up data from these 73 women revealed a similar prognosis in the two groups in terms of regression to normal, persistence of low grade disease, or progression to high grade CIN. Therefore, low grade cervical disease (ASCUS and CIN1) should be managed according to similar treatment protocols [Heatley 2001].

Assessment of cytologic follow-up for patients with atypical squamous cells of undetermined significance or low grade squamous intraepithelial lesions may be regarded as the standard recommended management. [Alanen et al 1998]

Long term follow-up of ASCUS

Until now, few studies have compared long-term follow-up and risk for invasive cancer in women with atypical squamous cells of undetermined significance (ASCUS). In a study by Raab et al [Rabb et al 1999], they conducted a 6-year review of pathology files for 651 women in whom ASCUS had been diagnosed in 1992. Data collected included patient demographics, follow-up diagnoses, time between follow-up examinations, and procedures performed. At follow-up, high-grade squamous intraepithelial lesions had developed in 9.0% of the women, and invasive cancer in none. Previous cervical history did not affect risk for an HSIL. Although the average time to first follow-up was 6.18 months, in 20.9% of the women the diagnosis of HSIL was not established until after 2.0 years. For individual pathologists, the percentage of HSILs ranged from 0% to 18.8%. Thus women with ASCUS who are followed up regularly are at low risk for development of invasive cancer [Rabb et al 1999].

Melinkow et al made a meta-analysis on the natural history of cervical squamous intraepithelial lesions to estimate rates of progression and regression without reatment [Melnikow et al 1998]. Eligible studies, representing 27,929 patients, were stratified according to entry cytologic findings. The following rates of progression to high-grade SIL at 24 months were found: ASCUS, 7.13% (95% confidence interval [CI] 0.8%,13.5%); low-grade SIL, 20.81% (6.08%, 35.55%); and high-grade SIL, 23.37% ,12.82%, 32.92%). The following rates of invasive cancer at 24 months were found: ASCUS, 0.25% (0%, 2.25%); low-grade SIL, 0.15% (0%, 0.71%); and high-grade SIL, 1.44% (0%, 3.95%). The following rates of regression to normal were found: ASCUS, 68.19% (57.51%, 78.86%); low-grade SIL, 47.39% (35.92%, 58.86%); and high-grade SIL, 35.03% (16.57%, 53.49%). Study heterogeneity was not explained by regression analysis of study level variables. Their findings for borderline and low-grade abnormal cervical cytologic results suggest a relatively low risk of invasive cervical cancer with observation up to 24 months and support the clinical policy of early colposcopy for high-grade lesions [Melnikow et al 1998].

ASCUS,CIN progress factors—unclear yet

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LOH of cervix neoplasms

Cervical carcinomas develop as a result of multiple genetic alterations, and specific alterations lead to specific clinical behavior. However, the effect of such alterations on the recurrence of cervical cancer after radiotherapy remains unknown.

A loss of heterozygosity (LOH), which points to a role for tumor suppressor genes (TSGs), oncogene amplification, and point mutations, are all thought to be involved, but there is as yet no complete picture of the relative roles for each of these genetic changes in patients with cervical carcinomas. To play a role in tumorigenesis, both copies of a TSG must be inactivated. The loss of one allele in a chromosome region may point to the presence of a TSG in that region. Chromosome arm 6p is one of those most frequently involved in a loss of heterozygosity in patients with cervical carcinoma. [Harima et al 2000]

Microsatellite instability

Human cancers progress through the accumulation of clonal genetic changes. Loss of heterozygosity has been demonstrated in almost all tumors analyzed to date and is easily detected by PCR-based microsatellite analysis. It has been demonstrated that appropriately selected microsatellite loci are commonly altered in many cancers and can serve as clonal markers for their detection. [Mao et al 1994] Using a panel of 13 microsatellites, we were able to detect 95% of transitional cell carcinomas of the urinary bladder by analysis of urine DNA. [Mao et al 1996] Furthermore, using a similar panel, 90% of bladder recurrences were detected prospectively. [Steiner et al 1997] In two cases, molecular changes preceded the clinical diagnosis of cancer.

Microsatellite instability of cervical cancer

In search of potential tumor suppresser genes (TSG), many LOH studies have been performed on primary cervical carcinomas. As in many other tumor types, cervical carcinoma displays frequent LOH at several loci, including those encompassing known tumor suppressor genes (TSG) [Rader et al 1996; Jones et al 1997; Mitra et al 1994; Mullokandov et al 1996; Kersemaekers et al 1998a]. One group of investigators evaluated 53 untreated primary cervical carcinomas at 57 loci and 49 (92.5%) tumors showed losses in 1 to 13 chromosomal arms.[Mitra et al. 1994] In a study by Rader *et al.*,[Reader et al. 1996] 80% of the tumors harbored at least one locus with chromosomal loss. The most common losses in these studies occurred on chromosomal arms 3p, 5p, 6p and 11q and helped us select our initial panel of markers to screen primary tumors. Consistent with previous findings, we noted LOH in more than 30% of primary tumors at many loci. These LOH studies also suggested that certain chromosomal losses might be early events in cervical carcinogenesis. Microsatellite abnormalities have been found at 3p, 5p, 9p and 11q in HSIL or carcinoma *in situ* (CIS) lesions, accompanying the invasive carcinoma.[Wistuba et al. 1997; Chung et al. 1992; Evans et al 1998; Mitra et al. 1995] Although it is less common, LOH has also been found in some low-grade (L)SIL.[Wistuba et al. 1997] These early losses were also detected in smears with few dysplastic cells, consistent with our observations in urine DNA from bladder